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New γ-Glutamylpeptides Isolated from the Seeds of Chives (*Allium schoenoprasum*)

N,N′-bis-(γ-glutamyl)-cystine, N,N′-bis-(γ-glutamyl)-3,3′-(2-methylethylene-1,2-dithio)-dialanine, γ-glutamyl-S-propylcysteine

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The amino acids and acid peptides which had been separated on an Amberlite IR-120 column from a 70 % ethanol extract of the ground seeds of chives were fractionated on a Dowex 1 × 8 column in acetate form. 1 kg of seeds was used at a time and each fraction was 20.3 ml/25 min. 1025 fractions were taken, 170 fractions with 0.5 N acetic acid, then 346 fractions with 1 N acetic acid, 284 fractions with 2 N acetic acid, and finally 225 fractions with 1 N hydrochloric acid. On the basis of the paper chromatograms obtained from the fractions, using butanol-acetic acid-water (12:3:5) as solvent and ninhydrin reagent, the fractionation proceeded as shown in Fig. 1. The amino acids emerged from the column in the 240 first fractions. At least most of the ninhydrin-positive substances in later fractions are γ-glutamylpeptides. The compounds denoted R XII, R XIII, R X, R XVIII, R XVII, and R XIX have so far been isolated in crystalline form, and the chemical structure of the four first ones mentioned has been elucidated. In this preliminary communication the results are reported on briefly.

Peptide R XII, γ-L-glutamyl-S-(prop-1-enyl)-L-cysteine. The isolation and structure of this compound is described in our earlier communications [1].

Peptide R XVIII, N, N′-bis-(γ-L-glutamyl)-3,3′-(2-methyl-ethylene-1,2-dithio)-dialanine. The peptide was isolated from fractions 750–823 (Fig. 1) and separated from peptides R XIII and R XIX on a cellulose powder column with isopropanol-acetic acid-water and crystallized from an acetone-water mixture. The fractions containing peptide R XVIII alone were evaporated to dryness *in vacuo*. On addition of acetone to its aqueous solution the peptide precipitated as a sirup. When ground with acetone, a white, solid substance was obtained which by paper chromatography was shown to be the pure peptide R XVIII. The yield was 590 mg/kg of seeds. After hydrolysis both with a preparation from calf kidney and in 1 N HCl two amino acids were found: L-glutamic acid ($[\alpha]_D^{22}$ + 34.8 in 6 N HCl) and an unknown sulphur-containing amino acid. After fractionation with butanol-acetic acid-water the unknown amino acid was crystallized by addition of acetone to its aqueous solution. When hydrogenated with Raney nickel as a catalyst, hydrogen was not consumed, but alanine was formed and a gas, which was found to be propane by mass spectrometry. The unknown amino acid could be identified as

$$HOOC \cdot CH(NH_2) \cdot CH_2 \cdot S \cdot CH_2 \cdot CH(CH_3) \cdot S \cdot CH_2 \cdot CH(NH_2) \cdot COOH$$

3,3′-(2-methylethyiene-1,2-dithio)-dialanine when this compound was synthesized. In three solvent systems the paper chromatograms of the natural and synthetic amino acids were identical. So were also the IR-spectra.

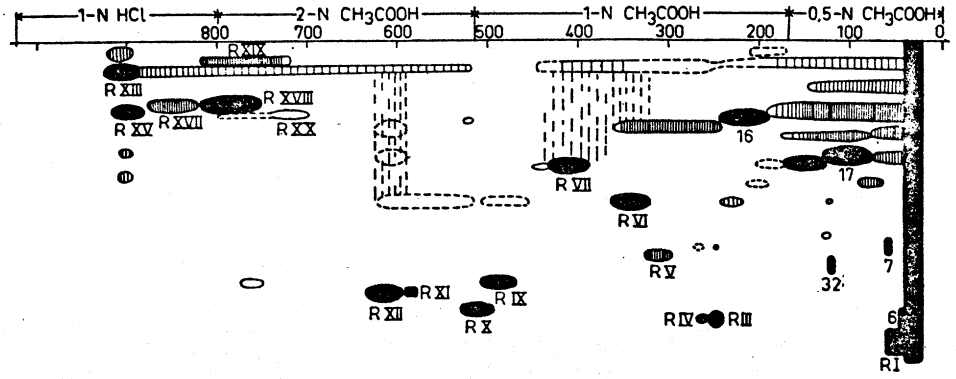


Fig. 1. Fractionation of ninhydrin-positive substances in the seeds of chives (*Allium schoenoprasum*) on a Dowex 1 × 8 column with acetic and hydrochloric acids (concentration of acids is given in the scheme). The fractions were studied by paper chromatography with butanol-acetic acid-water (12:3:5) as solvent. The spots coloured with ninhydrin are drawn on the basis of paper chromatograms. Some of the peptides may have been formed during isolation.

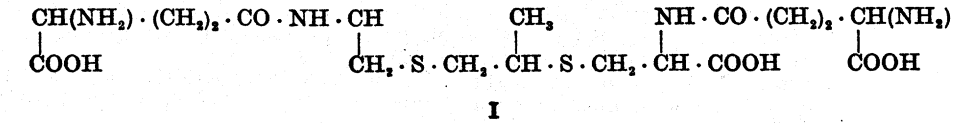
The peptide was then characterized by quantitative amino acid analysis, determination of γ-glutamyl bonds and end group analyses using both Sanger's method and deamination with nitric oxides, as N,N′-bis-(γ-L-glutamyl)-3,3′-(2-methylene-1,2-dithio)-dialanine (I).

The peptide is probably synthesized from γ-L-glutamyl-S-(prop-1-enyl)-cysteine (peptide XII) and γ-L-glutamyl-L-cysteine (peptide XIII in reduced form) (II).

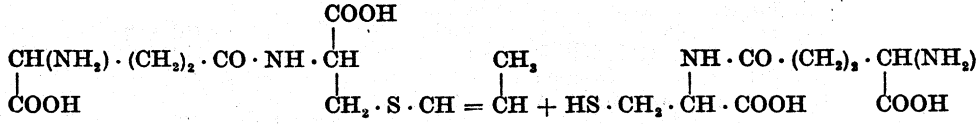
Peptide R XIII, N,N′-bis-(γ-glutamyl)-L-cystine. The peptide was isolated from fractions 880–927 (Fig. 1). Fractionation on a cellulose powder column with butanol-acetic acid-water led to pure peptide R XIII, which was crystallized from an acetone-water mixture. Yield 2.12 g/kg of seeds. In addition 0.5 g slightly impure R XIII was obtained from fractions 634–879.

L-Glutamic acid and L-cystine were formed when the peptide was hydrolyzed in different ways (with 6 N HCl, with Amberlite IR-120 in 70 % ethanol or enzymically with a preparation from calf kidney). The peptide was shown to contain a disulfide bond. Quantitative estimation of amino acids showed that the molar proportion glutamic acid: cystine was 2:1. The estimation of γ-glutamyl bonds and the end group estimations led to the result that the α-amino group of both glutamic acid residues in the peptide molecule are free. The results obtained indicate that peptide R XIII is N,N′-bis-(γ-L-glutamyl)-L-cystine.

$$HOOC-CH(NH_2)-CH_2-CH_2-CO-NH-CH(COOH)-CH_2-S-S-CH_2-CH(COOH)-NH-CO-CH_2-CH_2-CH(NH_2)-COOH$$



I



II

In the seeds it can partly be present in reduced form which is oxidized during the treatment of the plant material and isolation procedure

$HOOC-CH(NH_2)-CH_2-CH_2-CO-NH-CH(COOH)-CH_2-SH$
γ-L-glutamyl-L-cysteine

Peptide R X, γ-L-glutamyl-S-propyl-L-cysteine. This peptide was eluted from the Dowex 1 × 8 column partly in the same fractions as R IX (Fig. 1). They are poorly separated from each other also on the cellulose powder column and on the paper chromatogram. Peptide X was, however, isolated in pure form and its structure could be elucidated. It proved to be γ-L-glutamyl-S-propyl-L-cysteine. Because this peptide was earlier isolated in this laboratory [2] from garlic (*Allium sativum*) it is not treated in this preliminary communication.

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1. Virtanen, A. I. and Matikkala, E. J. *Suomen Kemistilehti B* **35** (1962) 2461; Matikkala, E. J. and Virtanen, A. I. *Acta Chem. Scand.* **16** (1962) 2461.
2. Virtanen, A. I., Hatanaka, M. and Berlin, M. *Suomen Kemistilehti B* **35** (1962) 52.

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